Parameter Identification of the Fermentative Production of Fructo-oligosaccharides by *Aureobasidium Pullulans*

R. Fekih-Salem
Faculty of Engineering, Automatic Control Laboratory
University of Mons
31 Boulevard Dolez, 7000 Mons, Belgium
& National Engineering School of Tunis, LAMSIN
University of Tunis El Manar
BP 37, Le Belvédère, 1002 Tunis, Tunisia
Email: radhouane.fekih-salem@umons.ac.be

A. Vande Wouwer
Faculty of Engineering, Automatic Control Laboratory
University of Mons
31 Boulevard Dolez, 7000 Mons, Belgium
Email: alain.vandewouwer@umons.ac.be

C. De Castro
Faculty of Engineering, Thermodynamique Department
University of Mons
31 Boulevard Dolez, Mons 7000, Belgium
Email: cristiana.castro@umons.ac.be

C. Nobre
University of Minho, Centre of Biological Engineering,
Campus de Gualtar, 4710-057 Braga, Portugal
Email: clarissenobre@deb.uminho.pt

A.-L. Hantson
Faculty of Engineering, Applied Chemistry and Biochemistry Department
University of Mons
56, Rue de l'Epargne, 7000 Mons, Belgium
Email: Anne-Lise.Hantson@umons.ac.be

***Abstract*—In this study, a mathematical model for the production of Fructo-oligosaccharides (FOS) by *Aureobasidium pullulans* is developed. This model contains a relatively large set of unknown parameters, and the identification problem is analyzed using simulation data, as well as experimental data. Batch experiments were not sufficiently informative to uniquely estimate all the unknown parameters, thus, additional experiments have to be achieved in fed-batch mode to supplement the missing information.**

***Index Terms*—Mathematical modeling, Parameter estimation, Fisher Information, Fructo-oligosaccharides, Biotechnology**

I. Introduction

Human health and well-being are mainly preserved by the metabolic activity of the bacterial community present in the gastrointestinal tract. Pre-, pro- and synbiotics can be used to control the intestinal function through modulation of microbiota composition and activity [10]. Because of their bifidogenic nature and their health-promoting properties, fructo-oligosaccharides (FOS), are classified as prebiotics. Functional properties as well as the technological potential of FOS make them widely attractive for food and pharmaceutical applications [7], [6], [1], [8].

Principal FOS include 1-kestose (GF_2), nystose (GF_3) and fructofuranosylnystose (GF_4) and can be naturally found in trace amounts in fruits, vegetables and honey [5]. Industrially, FOS can be produced from sucrose by β-fructofuranosidase enzymes with transfructosylating and hydrolytic activity, provided by fungi, such as Aureobasidium pullulans [4], [12], [15], [3]. FOS production yields can be affected by sucrose concentration in the medium as well as the amounts of small saccharides, such as glucose and fructose that can inhibit the fructosyltransferase enzymes and trigger FOS hydrolysis.

The maximization of the productivity, as well as the minimization of the small monosaccharides in the medium can be achieved by a tight process control. To this end, a dynamic model of the FOS production process is needed. The objective of the present study is to propose a macroscopic model of the bioprocess and to estimate the unknown parameters from experimental data. The parameter identification problem is however particularly challenging with regard to the large number of parameters. It is therefore required to collect data from dedicated experiments, achieved in batch and fed-batch mode, and carrying enough information on the reaction kinetics.

The paper is organized as follows. The FOS production process is modeled in section III. In Section IV, the methodology used for parameter identification is presented. Parameter estimation and model validation based on simulation data, as well as experimental data, are discussed in Section V. Finally, we draw conclusions in the last Section VI.

978-1-4799-8481-7/15/$31.00 ©2015 IEEE

II. Materials and methods

A. Bioreactor fermentations for FOS production

An inoculum of *Aureobasidium pullulans* was prepared by transferring 1 mL of spores suspension with $9\,10^7$ spores.mL^{-1} to a 500 mL Erlenmeyer flask with 100 mL of medium (100 g.L^{-1} sucrose, 0.5 g.L^{-1} KCl, 0.35 g.L^{-1} K_2SO_4, 0.5 g.L^{-1} $MgSO_4.7H_2O$, 0.01 g.L^{-1} $FeSO_4.7H_2O$, 5 g.L^{-1} $NaNO_3$ and 4 g.L^{-1} KH_2PO_4). The inoculum was grown at 28°C and 150 rpm and transferred after 3 days to a 5 L bioreactor - BIOSTAT® B module (Sartorius, Germany), with a working volume of 3 L of culture medium (200 g.L^{-1} sucrose and the same salt concentrations as the ones used in the inoculum). Bioreactor fermentations were carried out at 32°C and 385 rpm with a fixed pH of 5.5 .

B. Sugar analysis

The sugar analysis was performed according to [2], [9]. A HPLC (Jasco) equipped with a refractive index detector working at 30°C and a Prevail Carbohydrate ES 5u column (5 μm, 25 x 0.46 cm length x diameter) (Alltech) was used to analyze samples. The mobile phase consisted in a mixture of acetonitrile (HPLC Grade, Carlo Erba, France) in pure-water (70:30 v/v) with 0.04% of ammonium hydroxide in water (HPLC Grade from Sigma, Germany). Samples were eluted at 1 mL.min^{-1} flow-rate at room temperature. Chromatograms were further integrated using a Star Workstation software (Varian, USA). All the chemical standards used were of analytical grade.

III. Mathematical model

The FOS fermentative production model from Rocha et al. [11] is considered in the present study. The model includes the biomass growth rate equations and the enzymatic reactions. The enzymatic reactions are divided in hydrolysis reactions, representing FOS and sucrose degradation, and the transfructosylation reactions that describe FOS synthesis. The hydrolysis reactions of sucrose and FOS by the action of the enzyme are described by the following equations:

$$\begin{array}{rcl} GF & \xrightarrow{r_1} & k_1 G + k_2 F \\ GF_2 & \xrightarrow{r_2} & k_3 GF + k_4 F \\ GF_3 & \xrightarrow{r_3} & k_5 GF_2 + k_6 F \\ GF_4 & \xrightarrow{r_4} & k_7 GF_3 + k_8 F \end{array} \tag{1}$$

where GF, G, F and GF_i, $i = 2, 3, 4$, represent sucrose, glucose, fructose, 1-kestose, nystose and 1-fructofuranosyl nystose concentrations, respectively (gL^{-1}); r_i, $i = 1, \ldots, 4$, represents the hydrolysis rate ($gL^{-1}h^{-1}$) and k_i, $i = 1, \ldots, 8$, represents the pseudo-stoichiometric coefficient. For exemples, the pseudo-stoichiometric coefficient

$$k_1 = \frac{\text{Molecular weight of glucose}}{\text{Molecular weight of sucrose}} = \frac{180}{342} \tag{2}$$

is the conversion factor for grams of glucose produced from grams of sucrose consumed. All the other pseudo-stoichiometric coefficients were obtained using the same approach (see Appendix A). Note that Duan et al. [4] have considered the hydrolysis reaction of nystose GF_3 only.

The Transfructosylation reactions are given by

$$\begin{array}{rcl} 2GF & \xrightarrow{r_5} & k_9 GF_2 + k_{10} G \\ 2GF_2 & \xrightarrow{r_6} & k_{11} GF_3 + k_{12} GF \\ 2GF_3 & \xrightarrow{r_7} & k_{13} GF_4 + k_{14} GF_2 \end{array} \tag{3}$$

The biomass production reactions are given by

$$\begin{array}{rcl} Y_G G & \xrightarrow{r_8} & X \\ Y_F F & \xrightarrow{r_9} & X \end{array} \tag{4}$$

where X is the biomass concentration (gL^{-1}); Y_G and Y_F represent the biomass yield coefficient from glucose and fructose, respectively. Note that these yields are unknown since the molecular weight of X is unknown.

The model, which involves 7 state variables (concentrations) and 27 parameters, can be written as (in chemostat mode):

$$\left\{ \begin{array}{rcl} \dot{GF} & = & D(GF_{in} - GF) - r_1 + k_3 r_2 - r_5 + \frac{k_{12}}{2} r_6 \\ \dot{GF_2} & = & -r_2 + k_5 r_3 + \frac{k_9}{2} r_5 - r_6 + \frac{k_{14}}{2} r_7 - D.GF_2 \\ \dot{GF_3} & = & -r_3 + k_7 r_4 + \frac{k_{11}}{2} r_6 - r_7 - D.GF_3 \\ \dot{GF_4} & = & -r_4 + \frac{k_{13}}{2} r_7 - D.GF_4 \\ \dot{G} & = & k_1 r_1 + \frac{k_{10}}{2} r_5 - Y_G r_8 - D.G \\ \dot{F} & = & k_2 r_1 + k_4 r_2 + k_6 r_3 + k_8 r_4 - Y_F r_9 - D.F \\ \dot{X} & = & r_8 + r_9 - D.X \end{array} \right. \tag{5}$$

where GF_{in} and D represent the initial sucrose concentration ($g.L^{-1}$) and the dilution rate, respectively. The latter is given by $D = Q_{in}/V$ where Q_{in} and V represent the volumetric flow rate of sucrose feeding solution ($L.h^{-1}$) and the total volume of liquid inside reactor, respectively. The dynamical model of FOS production process (5) belongs to a large class of nonlinear bioprocess models and is referred as general dynamical state-space model of this class of bioprocesses [4], [11], [1], [6].

A possible structure of sucrose hydrolysis reaction rate is given in [4], [11] by the following Michaelis-Menten law:

$$r_1 = \frac{Vmh_{GF}\, GF}{Kmh_{GF} + GF}.$$

where Vmh_{GF} represents the maximum hydrolysis rate ($g.L^{-1}.h^{-1}$) and Kmh_{GF} represents the Michaelis-Menten constant for sucrose ($g.L^{-1}$).

The FOS hydrolysis kinetic equation is given by a modified Michaelis-Menten law describing the inhibition in enzyme-substrate reactions

$$r_i = \frac{Vmh_{GF_i}\, GF_i}{GF_i(1 + \frac{GF_i}{Kih_{GF_i}}) + Kmh_{GF_i}}, \quad i = 2, 3, 4,$$

where Vmh_{GF_i} represents the maximum hydrolysis rate ($g.L^{-1}.h^{-1}$), Kih_{GF_i} represents the substrate inhibition constant ($g.L^{-1}$), and Kmh_{GF_i} is the Michaelis-Menten constant ($g.L^{-1}$) for GF_i.

The sucrose transfructosylation kinetic equation is given by a modified Michaelis-Menten law describing the substrate inhibition and competitive glucose inhibition

$$r_5 = \frac{VmT_{GF}\ GF}{GF(1+\frac{GF}{Ksts}) + Kmst(1+\frac{G}{Kgst})}.$$

where VmT_{GF} is the maximum transfructosylation rate ($g.L^{-1}.h^{-1}$), $Ksts$ is the substrate inhibition constant ($g.L^{-1}$) for sucrose as a substrate, $Kgst$ is the competitive inhibition constant ($g.L^{-1}$) for glucose and $Kmst$ is the Michaelis-Menten constant ($g.L^{-1}$) for sucrose.

The modified Michaelis-Menten laws with competitive glucose inhibition for 1-kestose and nystose are given by

$$r_j = \frac{VmT_{GF_i}\ GF_i}{GF_i + Kmt_{GF_i}(1+\frac{G}{Kit_{GF_i}})}, \quad j = 6,7; \quad i = 2,3.$$

where VmT_{GF_i} is the maximum transfructosylation rate ($g.L^{-1}.h^{-1}$), Kmt_{GF_i} is the Michaelis-Menten constant ($g.L^{-1}$) for the GF_i oligosaccharide and Kit_{GF_i} is the competitive inhibition constant ($g.L^{-1}$) for glucose. The Monod laws are given by

$$r_j = \frac{\mu_{mj}\ S_j\ X}{S_j + K_{S_j}} \quad j = 8,9$$

where S_j ($j = 8,9$) are the glucose and fructose concentration ($g.L^{-1}$), respectively; μ_{mj} is the maximum specific growth rate (h^{-1}) for glucose and fructose; K_{S_j} is the affinity constant for the substrate ($g.L^{-1}$).

IV. Parameter identification

The solution of model (5) can be obtained through numerical integration (for instance using a ODE solver from Matlab) and depends on the parameter set θ

$$y_m(t,\theta) = f(\xi,\theta,t)$$

where $y_m(t,\theta) : \mathbb{R}_+ \times \mathbb{R}^{n_p} \to \mathbb{R}^{n_y}$ is the measurement vector (at first we assume that the full state vector is measured). The vector of data collected at time t_i is given by:

$$y(t_i) = y_m(t_i,\theta^*) + \eta_i, \quad i = 1,\dots,n_t$$

where θ^* represents the true value of the parameter vector; n_t represents the number of observation times. In addition, the measurement errors

$$\eta_i \hookrightarrow N(0,\Sigma), \quad i = 1,\dots,n_t$$

are assumed to be independent, zero mean and Gaussian. In practice, in the absence of a priori knowledge on the measurement error statistics, a pragmatic approach consists in using the Weighted Least Squares (WLS) criterion, where the cost function $J(\theta)$ is given by

$$J(\theta) = \sum_{i=1}^{n_t} [y(t_i) - y_m(t_i,\theta)]^T W^{-1} [y(t_i) - y_m(t_i,\theta)]$$

and

$$W = \begin{bmatrix} \max(y_m^1)^2 & 0 & \cdots & 0 \\ 0 & \max(y_m^2)^2 & \dots & 0 \\ \vdots & \vdots & \ddots & \vdots \\ 0 & 0 & \cdots & \max(y_m^{n_y})^2 \end{bmatrix}.$$

The covariance matrix of the measurement noise could be roughly estimated by $\hat{\Sigma} = \hat{\epsilon}^2 W$ where

$$\hat{\epsilon}^2 = \frac{J(\hat{\theta})}{n_y n_t - n_p},$$

and the WLS estimator

$$\hat{\theta} = \arg\min_{\theta} J(\theta)$$

is obtained using a combined procedure with the Quasi-Newton and Nelder-Mead methods (e.g. fminunc and fminsearch in MATLAB, respectively). The Fisher Information Matrix is determined by the following equation:

$$FIM = \sum_{i=1}^{n_t} [\frac{\partial y_m}{\partial \theta}]^T_{(t_i,\theta)} \hat{\Sigma}^{-1} [\frac{\partial y_m}{\partial \theta}]_{(t_i,\theta)}.$$

Assuming that the estimator is unbiased [14], the parameter covariance matrix can be approximated by

$$C_\theta \approx FIM^{-1}(\hat{\theta},\hat{\Sigma}).$$

Moreover, the standard deviation σ_j of the parameter estimates $\hat{\theta}_j$ can be obtained from the square root of the j^{th} diagonal element of C_θ

$$\sigma_j = \sqrt{C_{\theta_{jj}}}.$$

Finally, it is possible to estimate the confidence intervals for a given confidence level. In this work, 95% confidence level with a Gaussian distribution, was considered:

$$[\hat{\theta}_j - 2\sigma_j, \hat{\theta}_j + 2\sigma_j].$$

V. Numerical results

In this section, the parameter estimation problem is first explored in simulation. Batch and fed-batch experiments with different initial conditions and dilution rates are considered in order to collect enough informative data. Then, real-life experimental data are analyzed in the light of the previous results.

A. Simulation study

Since fungi grow in a heterogeneous form, it is difficult to measure biomass, and in the following it is therefore assumed that this information is not available. The following experimental field is chosen: three batch experiments (3B), three fed-batch (3FB), a combination of two experiments in batch and one in fed-batch (2B-FB), and a combination of two experiments in batch and two in fed-batch (2B-2FB).

The several experiments are carried out with different initial conditions of sucrose concentration, expressed in $g.L^{-1}$ (see Tab. I). The reference parameter vector $\hat{\theta}$ used to generate

the synthetic data are taken from the literature [4], [11] and is given in Table II. The measurement data is corrupted by additive Gaussian white noise with 5% relative error. To minimize the WLS criterion, a combination of algorithms are used, e.g., a Nelder-Mead method as implemented in the MATLAB function fminsearch is first used to approach the optimum, then a quasi-Newton algorithm as implemented in fminunc is used to refine the result.

The parameter estimation results are presented in Table II. Fig. 1 illustrates the good agreement between simulation data and the mathematical prediction in two batch and two fed-batch culture mode (2B-2FB).

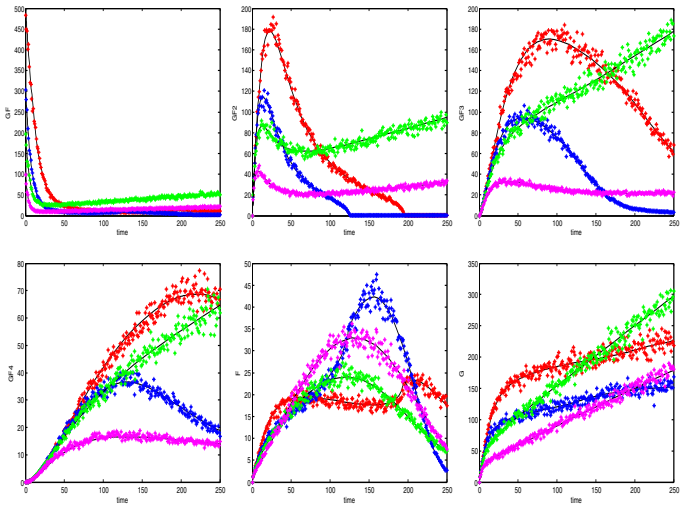

Figure 1. Direct Validation: Simulation data and the mathematical prediction in two batch and two fed-batch culture mode (2B-2FB).

An independent batch experiment with different initial conditions (in this case an initial sucrose concentration of 250 g/L) can be used for cross validation, showing satisfactory results (see Fig. 2 and Table II).

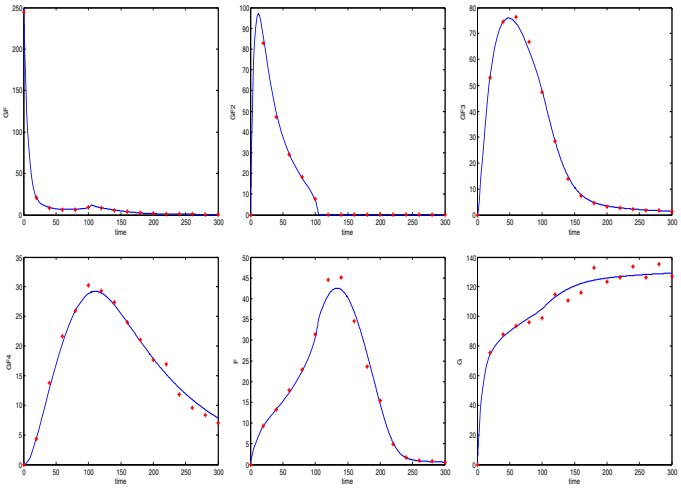

Figure 2. Cross validation with an independent batch experiment.

B. Experimental study

In real-life experiments, only batch experiments could be performed, and it is therefore a priori expected that the information content will not be enough to get accurate estimates.

First, only the data from 1 batch culture (called fermentation 1) is considered. In practice, not only the 27 kinetic parameters have to be considered, but also the initial concentrations, which are measured and are therefore uncertain. This leads to an identification problem with 34 parameters.

The direct validation checks that the model prediction is in agreement with the data used for identification and the cross validation checks that the model can reproduce previously unseen data, i.e., data that have not been used for identification. The direct validation results are shown in Fig. 3, and are quite satisfactory. However the confidence associated with the parameters is quite low.

In a next step, the data from 3 batch experiments (called fermentation 2, 3 and 4) are considered for parameter estimation and direct validation is shown in Fig. 4, while cross-validation (with an independent fermentation 5) is shown in Fig. 5, and Table IV.

The results are improved, but fed-batch experiments, as demonstrated in simulation, would be necessary to more significantly improve the estimates.

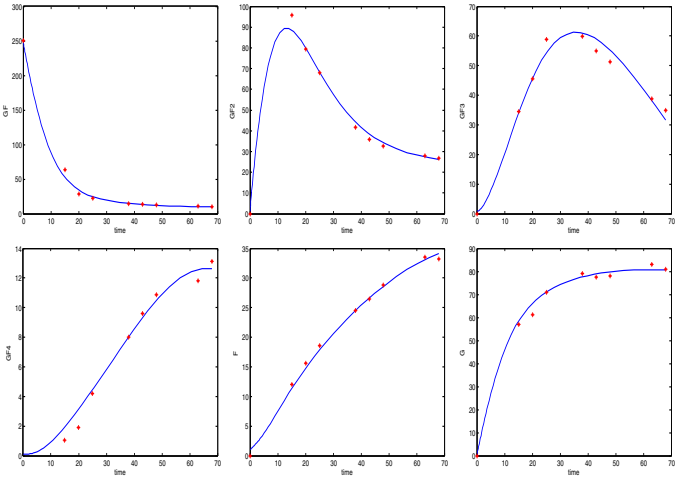

Figure 3. WLS method: Experimental data of the fermentation 1 and mathematical prediction.

VI. Discussion and Conclusion

A dynamic model of FOS production is presented from the cultivation of *Aureobasidium pullulans* fungi. The large number of parameters is a challenge for the identifiability analysis, and the experiment design. To tackle the identification problem, a parameter estimation code has been developed, based on the software IDEAS [13], including some extensions such as the possibility of handling data from several experiments at once. The numerical optimization procedure of the cost function is achieved using the Quasi-Newton and Nelder Mead methods. Parameter estimation results obtained

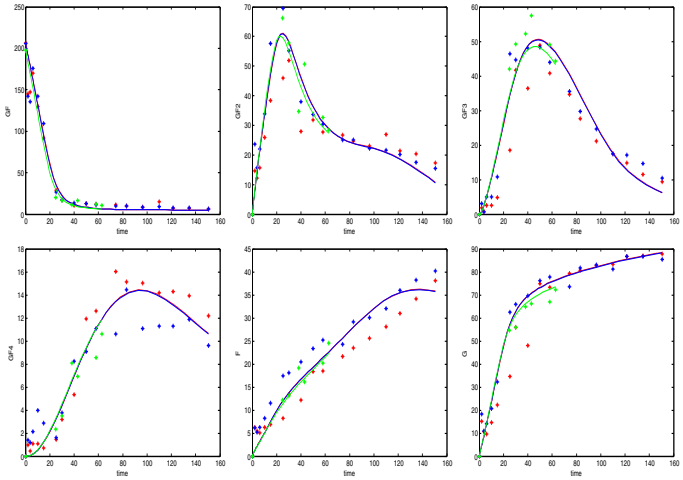

Figure 4. Direct Validation: Experimental data of three fermentations (2, 3 and 4).

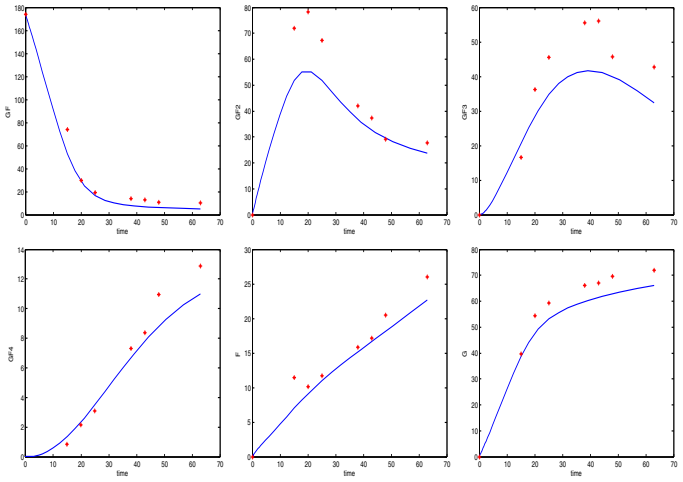

Figure 5. Cross Validation: Experimental data from fermentation 5 and mathematical prediction.

from experimental data collected in batch experiments are discussed.

The uncertainty of some parameters can be reduced by optimal experimental design. Our simulation study shows that the precision and accuracy of the parameter estimates can be improved by considering data collected in fed-batch experiments, and we therefore suggest a combination of experiments in batch and fed-batch culture modes.

Acknowledgment

This work presents research results of the Belgian Network DYSCO (Dynamical Systems, Control, and Optimization), funded by the Interuniversity Attraction Poles Programme initiated by the Belgian Science Policy Office. The authors thank the financial support from the F.R.S.-FNRS, the Belgium National Fund for the Scientific Research (Research Project 24643.08). C. Nobre thanks the Fundação para a Ciência e Tecnologia for the strategic funding of UID/BIO/04469/2013 unit.

References

[1] M. B. Alvarado-Huallanco and F. Maugeri-Filho. Kinetics and modeling of fructooligosaccharide synthesis by immobilized fructosyltransferase from *Rhodotorula sp*. *J Chem Technol Biotechnol*, 85:1654–1662, 2010.

[2] L. G. Dias, A. C. A. Veloso, D. M. Correia, O. Rocha, D. Torres, I. Rocha, L. R. Rodrigues, and A. M. Peres. UV spectrophotometry method for the monitoring of galacto-oligosaccharides production. *Food Chemistry*, 113(1):246–252, 2009.

[3] A. Dominguez, C. Nobre, L. R. Rodrigues, A. M. Peres, D. Torres, and I. Rocha. New improved method for fructooligosaccharides production by *Aureobasidium pullulans*. *Carbohydrate Polymers*, 89:1174–1179, 2012.

[4] K. J. Duan, J.S. Chen, and D. C. Sheu. Kinetic studies and mathematical model for enzymatic production of fructooligosaccharides from sucrose. *Enzyme Microb. Technol.*, 16:334–339, 1994.

[5] G.R. Gibson. Dietary modulation of the human gut microflora using prebiotics. *British Journal of Nutrition*, 80:209–212, 1998.

[6] F. Guio, L. D. Rugeles, S. E. Rojas, M. P. Palomino, M. C. Camargo, and O. F Sánchez. Kinetic modeling of fructooligosaccharide production using *Aspergillus oryzae* N74. *Appl Biochem Biotechnol*, 2012.

[7] T. Mutanda, M. P. Mokoena, A.O. Olaniran, B.S. Wilhelmi, and C.G. Whiteley. Microbial enzymatic production and applications of short-chain fructooligosaccharides and inulooligosaccharides: recent advances and current perspectives. *J Ind Microbiol Biotechnol*, 41(6):893–906, 2014.

[8] Koji Nishizawa, Mitsutoshi Nakajima, and Hiroshi Nabetani. Kinetic study on transfructosylation by b-fructofuranosidase from *Aspergillus niger* ATCC 20611 and availability of a membrane reactor for fructooligosaccharide production. *Food Sci. Technol. Res.*, 7 (1):39–44, 2001.

[9] C. Nobre, M. J. Santos, A. Dominguez, D. Torres, O. Rocha, A. M. Peres, I. Rocha, E. C. Ferreira, J. A. Teixeira, and L. R. Rodrigues. Comparison of adsorption equilibrium of fructose, glucose and sucrose on potassium gel-type and macroporous sodium ion-exchange resins. *Analytica Chimica Acta*, 654(1):71–76, 2009.

[10] V. De Preter, H. M. Hamer, K. Windey, and K. Verbeke. The impact of pre- and/or probiotics on human colonic metabolism: Does it affect human health? *Mol. Nutr. Food Res.*, 55:46–57, 2011.

[11] O. Rocha, C. Nobre, A. Dominguez, D.Torres, N. Faria, L. Rodrigues. J.A.Teixeira, E.C. Ferreira, and I. Rocha. A dynamical model for the fermentative production of fructooligosaccharides. In *10th International Symposium on process systems engineering*, pages 1–7, 2009.

[12] P. T. Sangeetha, M. N. Ramesh, and S. G. Prapulla. Recent trends in the microbial production, analysis and application of fructooligosaccharides. *Trends Food Sci Technol*, 16:442–457, 2005.

[13] R. Munoz Tamayo, B. Laroche, M. Leclerc, and E. Walter. IDEAS: a parameter identification toolbox with symbolic analysis of uncertainty and its application to biological modelling. In *15th IFAC Symposium on System Identification*, pages 1271–1276, 2009.

[14] E. Walter and L. Pronzato. *Identification of parametric models from experimental data*. Communications and Control Engineering. Springer-Verlag, Berlin, 1997, 1997.

[15] J. W. Yun and S. K. Song. The production of high-content fructo-oligosaccharides from sucrose by the mixed-enzyme system of fructo-syltransferase and glucose-oxidase. *Biotechnology Letters*, 15:573–576, 1993.

Appendix

The pseudo-stoichiometric coefficients can be calculated from the molecular weight of each state variable as (2):

$$k_1 = k_2 = k_{10} = \frac{180}{342}, \quad k_3 = k_{12} = \frac{1}{k_9} = \frac{342}{504}, \quad k_4 = \frac{180}{504},$$

$$k_5 = k_{14} = \frac{1}{k_{11}} = \frac{504}{666}, \quad k_6 = \frac{180}{666}, \quad k_7 = \frac{1}{k_{13}} = \frac{666}{828}, \quad k_8 = \frac{180}{828}.$$

	Batch B1	Batch B2	Batch B3	Fed-batch FB1	Fed-batch FB2	Fed-batch FB3
3B	400	300	200			
3FB				200	100	50
2B-FB	500	300		100		
2B-2FB	500	300		200	100	

Table I
INITIAL CONDITION OF SIMULATION DATA OF EACH EXPERIENCE IN DIFFERENT CULTURE MODE.

θ	Vmh_{GF}	Kmh_{GF}	Vmh_{GF_2}	Kih_{GF_2}	Kmh_{GF_2}	Vmh_{GF_3}
$\hat{\theta}$	1.43	111.57	7.58	2.72	0.61	7.97
3B	1.73±0.4	164.9 ± 79	6.3 ± 2.8	3.5 ± 1.9	0.68 ± 1.5	6.9 ± 4.4
3FB	1.72±1.1	173.6 ± 168	7.2 ± 2.1	3 ± 1	0.7 ± 0.9	9 ± 10
2B-FB	1.79±0.27	171 ± 49	7.4 ± 4.3	3 ± 2	0.7 ± 2.4	8.6 ± 8.6
2B-2FB	1.52±0.1	121.5 ±13.6	7.58 ± 4.1	2.8 ± 1.7	0.63 ± 2.2	8.7 ± 7.9
θ	Kih_{GF_3}	Kmh_{GF_3}	Vmh_{GF_4}	Kih_{GF_4}	Kmh_{GF_4}	Vmt_{GF}
$\hat{\theta}$	10.52	177.41	7.35	6.21	724.07	49.99
3B	12 ± 8.9	147.4 ± 108	8.8 ± 54	6.1± 40	899 ± 5637	59 ± 14
3FB	10.2 ± 13	211.5 ± 263	9 ± 79	6.3± 59	898 ± 7875	55 ± 16
2B-FB	9.3 ± 10.2	203 ± 226	8.4 ± 53	6.5± 44	870 ± 5688	52 ± 6.4
2B-2FB	9± 8.8	206.9 ± 205	9.4 ± 43.7	5.6± 28	982 ± 4625	64.7± 9
θ	$Ksts$	$Kmst$	$Kgst$	Vmt_{GF_2}	Kmt_{GF_2}	Kit_{GF_2}
$\hat{\theta}$	911.16	70.22	24.57	41.63	239.88	49.96
3B	593 ± 372	110 ± 48	35±9	50.7 ± 13.8	307 ± 96	52 ± 3
3FB	537 ± 682	84 ± 32	28±3.1	62.8 ± 29	397.8 ± 200	58 ± 5.2
2B-FB	891 ± 359	86 ± 19	30.6±4.2	58 ± 13	359 ± 91	51.5 ± 2
2B-2FB	482 ± 150	118 ± 27	33.1±3.7	69.2 ± 12.4	455 ± 90	55.8 ± 2.1
θ	Vmt_{GF_3}	Kmt_{GF_3}	Kit_{GF_3}	μ_{mG}	K_G	μ_{mF}
$\hat{\theta}$	11.53	333.07	49.95	$2.9e^{-5}$	397.98	0.0097
3B	13.8± 24	450 ± 746	59 ± 23	$3e^{-7}$ ± 126	$4e^{4}$ ±$22e^{12}$	0.01± e^{-3}
3FB	14 ± 22	439 ± 667	56 ± 20	$4e^{-6}$ ± $7e^{-3}$	1368 ± $2e^{6}$	0.01± $4e^{-4}$
2B-FB	12.7 ± 16	442 ± 565	64.8±16	$6e^{-11}$ ± $4e^{-3}$	258 ± $4e^{10}$	0.01± e^{-3}
2B-2FB	16 ± 13	536 ± 444	58 ±8.5	$4e^{-8}$ ± 0.3	$3e^{3}$ ± $3e^{10}$	e^{-3} ± $5e^{-4}$
θ	K_F	Y_G	Y_F			
$\hat{\theta}$	11.45	29.23	79.34			
3B	12.5 ±1.7	5.6 ± $6e^{6}$	78.9 ± 9.8			
3FB	14.3 ± 1	104 ± $5e^{4}$	79.7 ± 3.8			
2B-FB	12.3 ± 1.6	4.3 ± $2e^{8}$	76.7 ± 8.6			
2B-2FB	11.4 ± 1.3	11 ± $2e^{6}$	77.4 ± 4.7			

Table II
PARAMETER ESTIMATION FROM SIMULATION DATA IN BATCH AND FED-BATCH CULTURE MODE, WITH RELATIVE ERROR OF 5%.

Fermentation	1	2	3	4	5
$GF(0)$	250.29	197.51	206.59	205.94	173.96

Table III
INITIAL CONDITION OF $GF(0)$ FOR EXPERIMENTAL DATA OF EACH FERMENTATION IN BATCH CULTURE MODE.

Vmh_{GF}	Kmh_{GF}	Vmh_{GF_2}	Kih_{GF_2}	Kmh_{GF_2}	Vmh_{GF_3}	Kih_{GF_3}
1.3±16	234 ± 4812	2.3 ± 107	3.4 ± 196	0.6 ±113	9.6 ± 127	13.5 ± 228
Kmh_{GF_3}	Vmh_{GF_4}	Kih_{GF_4}	Kmh_{GF_4}	Vmt_{GF}	$Ksts$	$Kmst$
72.3 ± 1127	11 ± 43100	9.8±53965	943 ± $3.4e^{6}$	39 ± 9	48 ± 50	38 ± 473
$Kgst$	Vmt_{GF_2}	Kmt_{GF_2}	Kit_{GF_2}	Vmt_{GF_3}	Kmt_{GF_3}	Kit_{GF_3}
13.9±213	85 ± 1556	483 ± 9379	65 ± 132	23± 12888	854 ± 458706	49 ± 1729
μ_{mG}	K_G	μ_{mF}	K_F	Y_G	Y_F	
$3e^{-4}$± 1.3	22± 1341	0.004±1.3	8 ±240	363 ±$1.2e^{6}$	153 ± 42781	

Table IV
PARAMETER ESTIMATION FROM EXPERIMENTAL DATA OF THREE FERMENTATIONS (2, 3 AND 4) IN BATCH CULTURE MODE.

NOMENCLATURE

GF	Sucrose concentration (gL^{-1})
GF_2	1-kestose concentration (gL^{-1})
GF_3	Nystose concentration (gL^{-1})
GF_4	Fructofuranosylnystose concentration (gL^{-1})
F	Fructose concentration (gL^{-1})
G	Glucose concentration (gL^{-1})
X	Biomass concentration (gL^{-1})
Y_G	Biomass yield coefficient from glucose (gL^{-1})
Y_F	Biomass yield coefficient from fructose (gL^{-1})
Vmh_{GF}	Maximum hydrolysis rate for sucrose ($gL^{-1}h^{-1}$)
Kmh_{GF}	Michaelis-Menten constant for sucrose (gL^{-1})
Vmh_{GF_i}	Maximum hydrolysis rate for GF_i ($g.L^{-1}h^{-1}$)
Kih_{GF_i}	Substrate inhibition constant for GF_i (gL^{-1})
Kmh_{GF_i}	Michaelis-Menten constant for GF_i (gL^{-1})
VmT_{GF}	Maximum transfructosylation rate ($gL^{-1}h^{-1}$)
$Ksts$	Substrate inhibition constant for sucrose (gL^{-1})
$Kgst$	Competitive inhibition constant for glucose (gL^{-1})
$Kmst$	Michaelis-Menten constant for sucrose ($g.L^{-1}$)
VmT_{GF_i}	Maximum transfructosylation rate ($gL^{-1}h^{-1}$)
Kmt_{GF_i}	Michaelis-Menten constant for GF_i (gL^{-1})
Kit_{GF_i}	Competitive inhibition constant for glucose (gL^{-1})
μ_{mG}	Maximum specific growth rate for glucose (h^{-1})
K_G	Affinity constant for glucose (gL^{-1})
μ_{mF}	Maximum specific growth rate for fructose (h^{-1})
K_F	Affinity constant for fructose (gL^{-1})